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## Pharmacologic agents in the management of bleeding disorders

C.D. BOLAN AND B.M. ALVING

UNTIL RECENTLY, BLOOD products have served as the primary treatment for patients with mild congenital and acquired bleeding disorders. Despite intensive screening, unfractionated blood products continue to transmit infections that are potentially fatal.<sup>1-4</sup> The most frequently induced infection is non-A, non-B (NANB) hepatitis, which, by conservative estimates, is transmitted by 1 per 160 units of blood components.<sup>3</sup> Furthermore, human immunodeficiency virus (HIV) infection can be transmitted by as many as 1 per 40,000 units of seronegative components.<sup>1</sup> Screening measures have recently been instituted to limit the spread of another blood-borne viral infection, human T-cell lymphotropic virus type I (HTLV-I).<sup>4</sup>

The use of pharmacologic agents as alternatives to blood products has been stimulated by the desire to avoid these risks<sup>5</sup> and by their proven efficacy in patients with coagulopathies. Although recent reviews have focused on 1 deamino-8-D-arginine vasopressin or desmopressin (DDAVP) as a nontransfusional form of therapy,<sup>6,7</sup> agents such as vitamin K, estrogen, recombinant human erythropoietin, and the antifibrinolytic drugs tranexamic acid (AMCA) and epsilon-aminocaproic acid (EACA) may have essential roles in the management of common bleeding disorders. This review will discuss the pharmacology of these agents and present guidelines for their use.

DDAVP has been shown in controlled and uncontrolled trials to be effective in most patients with mild hemophilia A or von Willebrand's disease (vWD)<sup>8,9</sup> and in other patients with prolonged bleeding times.<sup>10</sup> AMCA and EACA are useful for patients with impaired hemostasis who are undergoing dental surgery.<sup>11-13</sup> Vitamin K can be used as primary treatment for the deficiency induced by some antibiotics,<sup>14,15</sup> excessive warfarin,<sup>16</sup> or the ingestion of long-acting warfarin-like compounds in rat poison.<sup>17,18</sup>

Pharmacologic agents may also improve hemostasis in patients with multifactorial bleeding disorders. This article will discuss the roles of DDAVP, estrogen, and recombinant human erythropoietin in patients with ure-

mia and the potential hemostatic effects of DDAVP as well as vitamin K in patients with cirrhosis. The use of DDAVP and aprotinin in patients undergoing cardiac bypass surgery will also be reviewed.

## Pharmacology of DDAVP

DDAVP is a synthetic analogue of vasopressin that was modified to produce antidiuretic action for patients with diabetes insipidus without inducing vasoactive side effects.<sup>6</sup> It has a plasma half-life of 124 minutes and is cleared by the liver and kidneys.<sup>19</sup>

DDAVP increases plasma factor VIII:c (FVIII:c) and von Willebrand factor (vWf) levels in normal subjects, in patients with mild to moderate hemophilia A, and in patients with vWD who have a mild to moderate decrease in vWf.<sup>20</sup> Through an unknown mechanism, the administration of DDAVP induces vWf to move from intracytoplasmic stores to the luminal surface of human endothelial cells.<sup>21</sup> The newly released vWf multimers, some of which have a higher molecular weight than those in preinfusion plasma,<sup>22</sup> increase platelet adherence.<sup>23</sup> This may explain the efficacy of DDAVP in patients with qualitative platelet disorders.

The increases in FVIII:c and vWf appear as early as 30 minutes after infusion of DDAVP, peak at 300 to 400 percent of baseline in 1 to 2 hours, and persist for 6 to 12 hours.<sup>24</sup> DDAVP also induces a transient fivefold to sevenfold increase in tissue-type plasminogen activator;<sup>25</sup> however, therapy with antifibrinolytic agents need not be given routinely to all patients receiving DDAVP.<sup>7</sup> Such agents are valuable for patients with mucosal bleeding or as prophylaxis for bleeding from dental work (see below).

DDAVP may lose its effect when doses are repeated at intervals of less than 24 hours<sup>26,27</sup> or when consecutive daily doses are given for more than a 2- to 4-day period.<sup>20,27</sup> Tachyphylaxis, which is probably due to the depletion of intracellular stores of vWf and FVIII:c, has been specifically described in patients with hemophilia,<sup>20</sup> uremia,<sup>28</sup> and vWD.<sup>29</sup> The efficacy of repeated infusions must be assessed on an individual basis, because the time at which tachyphylaxis occurs is not the same for all recipients.<sup>20</sup>

The extent of increase in FVIII:c and vWf induced by the intravenous infusion of DDAVP varies among individuals and even in the same individual who has received

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more than one injection.<sup>20</sup> For most recipients, the maximum increase occurs at a dose of 0.3  $\mu\text{g}$  per kg, which is administered intravenously during 30 minutes in 50 mL of normal saline for adults (10 mL for children who weigh less than 10 kg).<sup>30</sup> Although DDAVP is also efficacious when injected subcutaneously at that dose, a volume of 5 mL would be required for a 70-kg person, as the only concentration of DDAVP available for injection in the United States (US) is 4  $\mu\text{g}$  per mL. Such an injection might be uncomfortable<sup>31</sup> and can result in a diminished and delayed increase in FVIII:c or vWF when compared with the intravenous (IV) route of administration.<sup>24,32</sup>

DDAVP that is available for intranasal use in patients with diabetes insipidus is not as effective as intravenous DDAVP, even though the intranasal dose is 10 times that for IV administration (i.e., 2-4  $\mu\text{g}/\text{kg}$ ).<sup>33</sup> A disadvantage of intranasal use is that a 70-kg person requires approximately 2.8 mL of the solution, which has a DDAVP concentration of 100  $\mu\text{g}$  per mL. Some of this solution may inadvertently be swallowed and would therefore have diminished efficacy.

In Europe, more-concentrated preparations of DDAVP are available for subcutaneous and intranasal use (Table 1). Mannucci et al.<sup>24</sup> found that the rate and extent of increase in FVIII:c in patients with mild hemophilia who received a subcutaneous injection of DDAVP (0.5 mL of 40  $\mu\text{g}/\text{mL}$ ) was equivalent to that induced by the intravenous form (Table 1). A similar result was obtained in uremic patients.<sup>34</sup> Lethagen et al.<sup>30</sup> reported that a nasal spray of concentrated DDAVP yielded an increase in FVIII:c in healthy volunteers that was similar to that of an intravenous dose of 0.2  $\mu\text{g}$  per kg. Until these concentrated preparations are available in the US, the current products may play only a minor role in providing immediate home treatment for bleeding patients with mild hemophilia, vWD, or uremia.

Minor adverse effects of DDAVP consist of mild facial flushing and usually insignificant increases in pulse rate or decreases in blood pressure.<sup>7</sup> These side effects

may occur less frequently with subcutaneous or intranasal administration.<sup>31,32</sup> Rare but major adverse effects are hyponatremia and seizures, which have been described primarily in young children in the perioperative setting.<sup>9,35,36</sup> Seven patients ranging in age from 1 month to 8 years have developed hyponatremia 9 to 20 hours after administration of DDAVP; seizures occurred in six of the seven patients.<sup>9,35,36</sup> Predisposing factors for these reactions included emesis, overhydration with hypotonic fluid, and the infusion of multiple doses of DDAVP. In children and adults who receive DDAVP in the surgical setting, IV fluids, urine output, and serum electrolytes should be closely monitored during the subsequent 24 hours.

Other rare but major side effects are the development of myocardial infarction,<sup>37,38</sup> cerebral thrombosis,<sup>39</sup> or unstable angina<sup>40</sup> in recipients of DDAVP. In 1988, seven thrombotic events were recorded for 217,000 recipients.<sup>41</sup> Thus, the thrombotic potential for this drug is low; however, careful monitoring of recipients of DDAVP who have atherosclerosis is recommended.<sup>41</sup> These patients should not receive EACA or AMCA, which would block the fibrinolytic response to DDAVP.

#### Use of DDAVP in Patients with Hemophilia A

The efficacy of DDAVP in hemophiliacs is directly related to its ability to raise FVIII:c concentrations to levels that provide adequate hemostasis for surgery or bleeding episodes, that is, to about 30 to 50 percent of normal values for minor procedures or greater than 50 percent of normal for major surgery. Because DDAVP raises FVIII:c levels by 300 to 400 percent in 30 to 90 minutes after administration, it is an ideal therapy for patients who have mild hemophilia (FVIII:c levels from 6-40% of normal).<sup>9</sup> Although many patients with moderate hemophilia (FVIII:c activity 1-5% of normal) do not respond to DDAVP, some patients may have a response that is sufficient to provide temporary hemostasis.<sup>9</sup> An adequate increase in FVIII:c levels should be documented before surgery.

De la Fuente et al.<sup>9</sup> evaluated DDAVP as treatment for spontaneous bleeding episodes, including epistaxis, hemarthroses, and muscle hematomas in patients with mild or moderate hemophilia A. Clinical improvement, including cessation of bleeding and resolution of pain, occurred in 9 of 14 patients with mild hemophilia A and in 2 of 4 with moderate hemophilia after DDAVP therapy alone. An additional 16 of 17 patients with hemophilia A who received DDAVP as prophylaxis for procedures that included dental extraction and reconstructive palate surgery had no excessive bleeding. Patients who received DDAVP for dental procedures also received EACA concurrently.

Table 1. Effectiveness of DDAVP by route of administration

Route	DDAVP concentration $\mu\text{g}/\text{mL}$	Dose in $\mu\text{g}/\text{kg}$	Volume per 70 kg	Peak response Time (min)	FVIII:c ratio*
Preparations available in the United States					
Intravenous <sup>24</sup>	4	0.3-0.4	5cc†	30-60	3 - 4.5
Subcutaneous <sup>32</sup>	4	0.4	7cc	60	2.3
Intranasal <sup>33</sup>	100	2 - 4	2cc	90	1.4
Other preparations					
Subcutaneous <sup>24</sup>	40	0.3	0.5cc	69	3.75
Intranasal <sup>30</sup> (spray)	3000	4.0	0.1cc	60	2.7

\*Post-infusion activity/pre-infusion activity.

†Diluted in some normal saline.

Two patients who received DDAVP for treatment of a bleeding episode had a transient increase in bleeding after DDAVP treatment, despite a rise in FVIII:c activity as high as 87 percent of normal.<sup>9</sup> This effect may have been due to transient stimulation of fibrinolysis by DDAVP.

#### Use of DDAVP in vWD

DDAVP is effective therapy for most patients with vWD, a bleeding disorder that is heterogeneous in its clinical and laboratory manifestations.<sup>42</sup> Patients with vWD have either decreased levels of vWf antigen (vWf:Ag) with normal multimeric composition of the factor or normal or decreased levels of the antigen with loss of the higher-molecular-weight multimers. This results in excessive bleeding, usually in association with dental or surgical procedures, as a result of defective adherence of platelets to the subendothelium. Levels of FVIII:c may also be reduced.

Patients with vWD can be classified according to four major types on the basis of their plasma vWf levels and multimeric composition, as well as the sensitivity of their platelets to agglutination by ristocetin. Classification is a useful guide for determining therapy; for example, DDAVP is usually used only in patients with types I and IIA vWD.<sup>9</sup>

The majority of patients (80%) have type I vWD. In these patients, the vWf:Ag concentration is reduced; however, the multimeric structure is normal. Bleeding is caused by insufficient circulating levels of vWf and FVIII:c; most patients achieve excellent hemostasis after administration of DDAVP, which increases the concentration of vWf and induces the transient appearance of very high-molecular-weight multimers.

De la Fuente et al.<sup>9</sup> administered DDAVP to 13 patients with type I vWD. Eight of the 10 in whom a postinfusion bleeding time (BT) was measured had complete correction, and 2 had either partial correction or none. Eight of the 13 patients underwent dental or surgical procedures and required no blood-product support.

The response to DDAVP is variable in patients who have type IIA vWD, which is characterized by a low to normal vWf:Ag concentration and reduction or absence of the high- and medium-molecular-weight multimers in plasma.<sup>9,22,43</sup> In these patients, DDAVP may not induce the release of high-molecular-weight multimers. In the study by de la Fuente et al.,<sup>9</sup> only three of seven patients with type IIA vWD showed DDAVP-induced correction of the BT. Two of these patients received DDAVP before dental or surgical procedures and had excellent hemostasis, which indicates that DDAVP is beneficial in some patients with type IIA disease.

DDAVP is usually contraindicated for or ineffective in patients with type IIB vWD, which is characterized

by increased sensitivity of the platelets to ristocetin-induced agglutination and the absence of high-molecular-weight multimers in plasma.<sup>44</sup> In a recent study of nine patients with type IIB disease who received DDAVP, the following changes in BT were measured: one patient, complete correction; three patients, partial correction; three patients, no change; and two patients, further prolongation.<sup>44</sup> Platelet counts decreased in all patients from 12 to 84 percent of pretreatment levels. Fowler et al.<sup>45</sup> successfully used DDAVP in one patient with type IIB vWD; therefore, this agent may be efficacious in selected patients with this subtype.

Patients with type III vWD have a severe deficiency of vWf and FVIII:c and do not respond to DDAVP. For these patients, as well as those with type IIB vWD, cryoprecipitate or heat-treated FVIII concentrates that have functional vWf are indicated.<sup>46,47</sup>

#### Use of DDAVP in Patients with Acquired vWD

Patients with underlying illnesses such as myeloproliferative syndromes, lymphoproliferative or autoimmune disorders, or glycogen storage disease type Ia may acquire vWD.<sup>48-50</sup> DDAVP produces a shorter duration of response (<4 hours) for patients with acquired vWD.<sup>48</sup> However, in two patients with myeloma and vWD, DDAVP has been used successfully to control gingival bleeding and to provide prophylaxis for tooth extractions.<sup>49</sup> For patients who are refractory to DDAVP or cryoprecipitate, high-dose intravenous immune globulin may be efficacious.<sup>51</sup>

#### Use of DDAVP for Patients with Abnormal Platelet Function or Isolated Prolongation of the BT

Kobrinisky et al.<sup>10</sup> first documented the efficacy of DDAVP in patients who had prolongation of the BT but no evidence of vWD. Platelet studies demonstrated one of the following patterns: normal or abnormal response to aggregating agents or a storage-pool defect. Those patients with a prolonged BT and normal aggregation studies were classified as having an isolated prolongation of the BT. Six additional reports have confirmed the efficacy of DDAVP in these groups of patients (Table 2).<sup>52-57</sup>

Two studies have examined the effectiveness of DDAVP in patients who have ingested aspirin. In a controlled trial, Mannucci et al.<sup>52</sup> found that DDAVP significantly shortened the BT in six healthy volunteers who had ingested a single 500-mg dose of aspirin. However, the BT in these volunteers was only minimally elevated after the aspirin ingestion. In another study,<sup>10</sup> DDAVP corrected the prolonged BT of two aspirin-treated patients who then underwent invasive procedures without

Table 2. DDAVP for patients with prolonged bleeding times\*

Laboratory diagnosis	Number of patients	Bleeding time (mins)	
		Shorten (%)	Normalize (%)
<b>Responders</b>			
Unspecified platelet defect <sup>53,54</sup>	20	20 (100)	17 (85)
Isolated prolongation of bleeding time <sup>32,54,56</sup>	38	34 (89)	30 (79)
Storage pool defect <sup>50,54,56</sup>	33	25 (75)	8 (25)
<b>Nonresponders†</b>			
Glanzmann's <sup>10,52,54</sup> thrombasthenia	4	0 (0)	0 (0)
Thrombocytopenia <sup>52</sup>	15	0 (0)	0 (0)

\*Excludes vWD and drug ingestion.

†No significant shortening.

excessive blood loss. Additional studies are needed to define the role for DDAVP in this clinical setting.

There are at least two groups of patients for whom DDAVP is not effective (Table 2). When infused into 15 patients with thrombocytopenia, DDAVP caused no significant change in the BT, which was initially greater than 30 minutes.<sup>52</sup> DDAVP also failed to shorten BT in four patients with Glanzmann's thrombasthenia, an inherited disorder characterized by the absence of the platelet glycoprotein IIb/IIIa.<sup>10,52,54</sup> Antifibrinolytic agents, however, are efficacious in patients with Glanzmann's thrombasthenia<sup>58,59</sup> as well as those with thrombocytopenia (see below).

#### Antifibrinolytic Agents

Systemic or local fibrinolysis occurs when tissue-type plasminogen activator, which is released from the endothelial cells, converts plasminogen to plasmin in the presence of fibrin.<sup>60,61</sup> The plasmin then degrades the fibrin clot. Both plasminogen and plasmin bind to fibrin through their lysine-binding sites. In the presence of the lysine analogues EACA and AMCA, the binding is reduced and fibrinolysis is delayed.<sup>11</sup>

The clinical use of lysine analogues began with EACA in the early 1960s. EACA and now AMCA are currently used locally or systemically in selected patients who may benefit from a reduction in fibrinolytic activity.

Both EACA and AMCA are well absorbed orally and are cleared virtually unchanged by the kidneys.<sup>11</sup> They are distributed widely throughout the body. AMCA crosses into cerebrospinal fluid, semen, synovial fluid, and cord blood (through the placenta).

AMCA is more potent than EACA on a molar basis and can be given to adults at a dose of 1 g every 6 hours, intravenously or orally. The highest recommended dose for EACA is 100 mg per kg given orally every 6 hours or, when infused intravenously, 10 g followed by 1 g

per hour;<sup>11,62</sup> however, lower doses of 6 to 8 g per day have frequently been used in clinical studies. The half-life is between 1 and 2 hours for both analogues in patients with normal renal function.<sup>11</sup> The dose must be reduced for patients with renal failure.<sup>11,62</sup>

The most common side effects for AMCA and EACA, which are dose dependent, include nausea, cramping, and diarrhea.<sup>11,62</sup> Myopathy and myonecrosis are rare complications associated with long-term use of high-dose EACA.<sup>63</sup> These side effects may be reduced or delayed with AMCA.<sup>63</sup> Baseline and serial muscle enzymes should be monitored if patients are receiving long-term therapy.

A potentially fatal side effect of antifibrinolytic agents is thrombosis. One study<sup>64</sup> demonstrated a significant decrease in repeat bleeding in patients with subarachnoid hemorrhage who received AMCA. However, there was no decrease in mortality because of a concomitant increase in cerebral infarction. An increased incidence of thrombosis has not been found in patients who received EACA within the first 24 hours after prostatectomy,<sup>65,66</sup> or cardiac bypass.<sup>67</sup> The use of low doses of EACA (6-8 g/day) in patients with sickle cell disease and hematuria has also not resulted in thrombosis.<sup>68</sup> Hemophiliacs who receive EACA for hematuria develop intrarenal clots with the same frequency as those who are untreated; nonetheless, EACA is not recommended for treatment of hematuria in these patients unless they are having significant blood loss.<sup>69</sup>

#### Use of Antifibrinolytic Agents in Patients Undergoing Oral Surgery

Saliva contains plasminogen activators, and excessive bleeding frequently complicates oral surgery in patients with hemophilia or other coagulopathies. Antifibrinolytic drugs have a well-established role in improving hemostasis in these patients. In a double-blind study, hemophiliacs who were undergoing dental extractions received oral AMCA at a dose of 1 g three times a day. All patients received an initial transfusion of factor concentrate. Only 2 of 14 AMCA-treated patients required further transfusions, as compared with 11 of 14 patients who did not receive AMCA.<sup>12</sup>

Recently, Sindet-Pedersen<sup>70</sup> showed that AMCA administered systemically is poorly distributed to saliva. However, therapeutic levels are present in saliva for more than 2 hours when AMCA is provided as a mouthwash; furthermore, systemic absorption is minimal.<sup>70</sup> Sindet-Pedersen et al.<sup>71</sup> used AMCA orally and as a mouthwash, during and after oral surgery, to provide hemostasis in patients with hemophilia and vWD.

In a recent double-blind, placebo-controlled study of warfarin-treated patients, AMCA mouthwashes reduced mucosal bleeding during and after oral surgery even though the patients were maintained on systemic anticoagula-

tion.<sup>13</sup> Bleeding episodes occurred in only 1 of 19 AMCA-treated patients, as compared with 8 of 20 untreated patients. Treated patients received 10 mL of a 4.8 percent AMCA solution, taken as a mouthwash for 2 minutes four times a day for 7 days.<sup>13</sup>

#### Treatment of Disseminated Intravascular Coagulation in Patients with Acute Promyelocytic Leukemia

Patients with acute promyelocytic leukemia may develop laboratory and clinical evidence of disseminated intravascular coagulation (DIC).<sup>72</sup> Standard coagulation assays do not reliably predict the potential for hemorrhage; however, patients who are anemic, thrombocytopenic, elderly, or who have a high percentage of blast cells are at increased risk for bleeding.<sup>72</sup> The coagulopathy may be initiated by promyelocyte-derived tissue factor, which promotes thrombin generation, followed by fibrin formation and a subsequent fibrinolytic response.<sup>73</sup> Fibrinolysis may also be induced by urokinase released from the promyelocytes.<sup>74</sup>

Retrospective studies have reported a decrease from 28 to 19 percent in the hemorrhagic deaths of patients who receive heparin.<sup>75</sup> Presumably, heparin, in doses ranging from 300 to 700 U per hour, prevents the onset of DIC in patients by inhibiting thrombin.<sup>76</sup>

Recently, Schwartz et al.<sup>77</sup> showed that EACA, in combination with heparin may improve hemostasis in patients with acute promyelocytic leukemia. They reported that bleeding occurred in three of four patients in whom  $\alpha_2$ -antiplasmin, the major inhibitor of plasmin, had been depleted to less than 30 percent of normal. Treatment of four patients with EACA (1 g/hr by continuous IV infusion) resulted in increases of fibrinogen levels within 24 hours and in cessation of bleeding in the two patients who were symptomatic. All patients continued to receive heparin at a dose of 500 to 750 U per hour.

In a double-blind study of patients with acute promyelocytic leukemia, Avvisati et al.<sup>78</sup> found that AMCA administered at a dose of 6 g daily by continuous IV infusion reduced the transfusion of red cells and platelets by 50 percent or more. None of the patients received heparin and there were no thrombotic complications.

#### Antifibrinolytic Agents in Patients with Thrombocytopenia

Patients with prolonged periods of megakaryocytic thrombocytopenia secondary to chemotherapy, myelofibrosis, leukemia, myelodysplastic syndromes, and aplastic anemia have been treated with EACA for prophylaxis and control of bleeding.<sup>79-81</sup> In one uncontrolled study,<sup>79</sup> bleeding that was primarily of oral mucosal origin was stopped in 14 of 15 thrombocytopenic patients

who received EACA at a dose of 4 g every 6 hours during an acute bleeding episode, followed by 3 to 4 g every 8 hours after hemostasis was achieved. Platelet counts were unaffected, yet transfusion requirements in four patients who received long-term EACA were reduced from an average of 95 units of platelets per month to 4 units per month. Similar results were reported by Bartholomew et al.<sup>30</sup> in patients with immune and non-immune thrombocytopenia who received 4 to 6 g of EACA daily in divided doses. Garewal and Durie<sup>81</sup> also confirmed a marked reduction in oral mucosal bleeding in thrombocytopenic patients who received EACA. There were no complications of thrombosis or myopathy in any of these studies.

#### Pharmacology of Vitamin K

Vitamin K is a necessary cofactor for the carboxylation of the glutamic acid residues of multiple proteins, including clotting factors II, VII, IX, and X. The carboxylated factors exert coagulant activity by interaction with calcium, which in turn binds them to phospholipid surfaces. During the carboxylation reaction, the active or reduced form of vitamin K is converted to an epoxide that must undergo reduction in order for the carboxylation cycle to be repeated.<sup>82</sup> Warfarin and its analogues, as well as certain antibiotics, inhibit the epoxide reductase and impair the regeneration of active vitamin K. Replacement of vitamin K restores the carboxylation reaction and regenerates functional clotting factors.

Although vitamin K occurs in several forms, only vitamin K<sub>1</sub> (phylloquinone) from leafy plants and vegetables is in clinical use.<sup>82</sup> Vitamin K<sub>2</sub> (menaquinone), which is produced by bacteria, including the normal flora of the human intestine, has an undetermined role in the physiologic regulation of vitamin K metabolism. The clinical use of vitamin K<sub>3</sub> (menadione) is limited by its side effects, which include hemolytic anemia and liver damage.<sup>82</sup> Anaphylactic reactions with vitamin K have not occurred with slow infusion of intravenous preparations.<sup>83</sup>

#### Use of Vitamin K in Warfarin-Treated Patients

The anticoagulant effect of warfarin can be potentiated by dietary vitamin K deficiency or by medications such as cimetidine<sup>84</sup> or trimethoprim-sulfamethoxazole.<sup>85</sup> The latter drug, as well as metronidazole, can cause severe hypoprothrombinemia by decreasing the metabolism of the more potent S-warfarin isomer without changing overall plasma warfarin levels.<sup>85,86</sup> Small doses (1-4 mg) of intravenous or subcutaneous vitamin K alone can be used to treat severe hypoprothrombinemia and restore therapeutic anticoagulation in patients who are not actively bleeding.

In one study,<sup>16</sup> seven patients with a bleeding diathesis and marked prolongation of the prothrombin time (PT) due to excessive warfarin received 1 of mg vitamin K intravenously (Table 3). The prolonged PT decreased to therapeutic or subtherapeutic levels in five patients after a single dose and in an additional patient after a second infusion of vitamin K at a dose of 2 mg. A seventh patient received only one infusion because of initial vasodilatation. There were no thromboembolic events in these patients, all of whom had prosthetic valves.<sup>16</sup>

Small doses of vitamin K are especially useful in therapeutically anticoagulated patients who are to undergo invasive procedures. The PT may remain elevated more than 72 hours after cessation of warfarin. However, infusion of 1 mg of vitamin K can normalize the PT within 24 hours, and it can remain in the normal range for up to 72 hours.<sup>87</sup>

#### Treatment of Anticoagulation Induced by Rat Poison

Patients who have ingested rat poison may initially have a markedly prolonged PT and activated partial thromboplastin time (APTT) as well as a bleeding diathesis.<sup>17,18</sup> The anticoagulants in the poison, which are known as superwarfarins (difenacoum or brodifacoum), act in a fashion similar to warfarin. That is, they induce a deficiency of functional vitamin K by inhibiting the enzymes that reduce vitamin K epoxide to its active form. Superwarfarins differ in several respects from warfarin. A single dose in humans can prolong anticoagulation for as long as 7 weeks; thus, patients must receive extended treatment with vitamin K.<sup>17,18</sup> The superwarfarins are much more potent than warfarin and high doses of vitamin K are required to overcome the anticoagulant effect (usually 25-50 mg administered orally twice a

day).<sup>17,18</sup> The transfusion of fresh-frozen plasma (FFP) or factor IX concentrate will only transiently correct the abnormalities in these patients. The PT is the best guide for treatment. These compounds cannot be detected with the usual assays that measure warfarin; assays that specifically measure superwarfarins are required.

#### Treatment of Antibiotic-Induced Vitamin K Deficiency

Antibiotics can impair hemostasis by causing platelet dysfunction<sup>88</sup> and possibly by reducing vitamin K levels through disruption of the endogenous gut flora that produce vitamin K.<sup>14</sup> Cephalosporins containing the N-methylthiotetrazole side chain can induce hypoprothrombinemia and clinically significant bleeding by exerting a warfarin-like effect in vivo.<sup>15</sup> Agents with this side chain include cefamandole, moxalactam, cefoperazone, cemetazole, cefotetan, and cefmenoxime. Patients who are debilitated, who are uremic or receiving nonsteroidal agents or warfarin are at increased risk for bleeding.<sup>15</sup> Intravenous vitamin K in doses of 1 to 2 mg may be effective within 24 to 36 hours, although some patients may require higher doses. Prophylactic vitamin K, administered at a dose of 10 mg subcutaneously twice a week, is indicated for debilitated patients. Because moxalactam may cause significant platelet dysfunction, bleeding caused by this agent may not respond to vitamin K alone.<sup>88</sup>

#### Treatment of Vitamin K Deficiency

Previously unsuspected vitamin K deficiency is diagnosed frequently in critically ill hospitalized patients, as well as in patients who have poor nutrition or malabsorption. In one study of 42 hospitalized patients who had a prolonged PT or APTT, the abnormalities were corrected within 12 hours after vitamin K therapy (20-25 mg of vitamin K given in 25 mL of normal saline solution intravenously over 30 minutes).<sup>83</sup> All of the patients were receiving antibiotics, although only one-half were taking cephalosporins. Other risk factors included recent surgery (50%), reduced dietary intake (62%), an intensive-care-unit setting (57%), and renal failure (38%). After the initial correction of the prolonged PT, patients were given vitamin K (5 mg two or three times a week) as prophylaxis against further deficiency.

#### Treatment of Complex Coagulopathies

##### Uremia

Patients with uremia frequently experience bleeding secondary to an acquired platelet dysfunction potentiated by severe anemia.<sup>89</sup> Platelets from uremic patients have multiple abnormalities, including a storage pool defect,<sup>90</sup> and when studied in an in vitro system, they show

Table 3. Reduction of prothrombin time\* by intravenous vitamin K in seven patients

Admission prothrombin time (sec)	Dose of vitamin K (mg)	After vitamin K prothrombin time (sec)	Hours after vitamin K
1. 29.8	1	21.3	15
2. 80.0	1	56.7	15
		28.6	76
3. 39.1	1	17.5	14
4. 29.4	1	14.9	10
5. 38.6	1	34.1	12
	2	12.4	18
6. 27.8	1	16.2	21
7. 31.0	0.5	21.0	12

\*Control prothrombin times for all patients ranged from 10 to 13 seconds.

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decreased adhesiveness to human vessel subendothelium.<sup>91</sup> Gralnick et al.<sup>92</sup> recently reported that the platelets contain decreased vWf and that the high-molecular-weight forms of vWf are relatively decreased in plasma from uremic patients.

In uremia the degree of prolongation of BT is the test that correlates best with the likelihood of clinical bleeding.<sup>93</sup> Thus, the success of hemostatic agents in uremic patients is measured by their effect on BT as well as by their ability to induce hemostasis during surgical procedures such as renal biopsies.

Although hemodialysis can induce partial correction of bleeding diathesis,<sup>90</sup> hemostatic agents such as DDAVP and conjugated estrogens can be used to induce complete correction of prolonged BT (Table 4).

DDAVP acts within 1 to 2 hours for short-term control of bleeding, probably by promoting platelet adhesion through increasing the concentration of high-molecular-weight multimers of vWf.<sup>94</sup> In a double-blind, randomized, placebo-controlled trial, 12 patients showed partial correction of prolonged BT lasting at least 4 hours after the IV administration of DDAVP (0.3 µg/kg).<sup>94</sup> Nine patients then underwent invasive procedures, including renal biopsy and nephrectomy, with no excessive bleeding.

The effect of estrogen on correcting prolonged BT in uremic patients was first reported by Liu et al.<sup>95</sup> in 1984. This report was followed by a double-blind randomized trial in which six uremic patients received IV conjugated estrogens at a dose of 0.6 mg per kg per day for 5 days.<sup>96</sup> The prolonged BT was corrected partially by 6 to 48 hours after the first dose. The peak effect was at 5 to 7 days and lasted for as long as 14 days. The mechanism of action of estrogens is not known.<sup>96</sup> The efficacy of more prolonged treatment<sup>97</sup> or of oral doses has not been established. Lower doses of IV estrogens (0.3 mg/kg) are not effective.<sup>97</sup> For patients who do not respond to DDAVP or estrogens, cryoprecipitate may induce a correction of prolonged BT.<sup>98</sup>

Increasing the hematocrit in anemic patients to 30 percent shortens the BT,<sup>99</sup> which may be explained by the

observation that an elevated red cell volume increases radial migration of platelets to the vessel wall.<sup>100</sup> The administration of erythropoietin to uremic patients induces a similar reduction in BT by increasing the red cell mass.<sup>101</sup> Moia et al.<sup>101</sup> recommended that uremic patients who are being treated with erythropoietin should target a hematocrit of 30 percent as a goal of therapy, because higher values may be associated with an increased incidence of thrombotic complications.

Moderate doses of aspirin<sup>102</sup> or drugs such as diphenhydramine and diazepam<sup>93</sup> that have minimal platelet inhibitory properties in normal individuals can markedly prolong BT in uremic patients. In 12 of 29 uremic patients who received 100 mg of aspirin, BT increased from initial values of less than 7 minutes to values of more than 15 minutes 1 hour later.<sup>102</sup> The same dose given to healthy controls did not significantly prolong BT. A careful drug history, adjustment of the dose for the degree of renal failure, and discontinuation of unnecessary medications are therefore important aspects of treatment of bleeding patients with uremia.

#### Cirrhosis

The coagulopathy associated with cirrhosis is often the result of multiple hemostatic defects.<sup>103,104</sup> Thrombocytopenia,<sup>104</sup> defective platelet aggregation,<sup>105</sup> increased fibrinolysis,<sup>106</sup> decreased levels of coagulation factors,<sup>107</sup> defective vitamin K-dependent carboxylation,<sup>108</sup> impaired ristocetin cofactor activity,<sup>109</sup> and chronic DIC<sup>110</sup> have all been described. Patients may also have an acquired dysfibrinogenemia due to increased sialic content of the fibrinogen molecule; however, this abnormality is not associated with clinical bleeding.<sup>111</sup> The use of blood products in cirrhotic patients is often unsatisfactory; transfused platelets may be sequestered in the spleen,<sup>104</sup> factor concentrates may induce thrombosis,<sup>112</sup> and FFP must be transfused in large volumes to maintain even a transient hemostatic effect.<sup>113</sup>

DDAVP and vitamin K each augment hemostasis in cirrhosis and may be useful in the control of bleeding or

Table 4. Therapeutic options for treatment or prophylaxis of uremic bleeding

Option	Dose	Effect		
		Onset	Peak	Duration
DDAVP <sup>94,94</sup> (intravenous or subcutaneous)	0.3 µg/kg	1 hour	1-4 hours	4-12 hours
Estrogens <sup>96,97</sup>	0.6 mg/kg/day (intravenous for 5 days)	6 hours	5-7 days	~ 14 days
Erythropoietin <sup>101</sup>	Hct 26-30%*	Days	Days to weeks	Indefinite
Red cell transfusions <sup>99</sup>	Hct 26-30%*	Immediate	Immediate	Indefinite
Cryoprecipitate <sup>98</sup>	10 units	1-4 hours	1-12 hours	<24 hours
Discontinuation of medications <sup>93,102</sup>			Hours to days	

\*Elevation of the hematocrit (Hct) to 26 to 30 percent.

as prophylaxis before invasive procedures. Two double-blind randomized clinical trials have demonstrated the efficacy of DDAVP at standard doses in shortening BT in patients with cirrhosis.<sup>52,114</sup> No invasive procedures were done in either trial. Correction of prolonged BT was partial and transient,<sup>52</sup> and further clinical trials are necessary to determine the appropriate clinical role of DDAVP in cirrhosis.

Vitamin K has an integral role in the therapy of the coagulopathy of cirrhosis.<sup>103,112,114</sup> It has been given routinely in many studies as prophylaxis before liver biopsy; blood products were then used for patients in whom the PT and APTT did not normalize after 3 days of therapy. Although vitamin K does not reverse the acquired vitamin K carboxylase deficiency of cirrhosis,<sup>108</sup> patients may still have correction of their prolonged PT or APTT after treatment with vitamin K; in one study,<sup>115</sup> correction of the PT to acceptable levels occurred in 37 percent of patients with cirrhosis after 3 days of therapy.<sup>115</sup>

Antifibrinolytic agents are not currently used to treat bleeding in patients with cirrhosis and have been associated with thrombosis in this clinical setting.<sup>116</sup>

DDAVP may be useful as prophylactic and interventional therapy (in combination with other agents as appropriate) for those patients with prolonged BT, as there is frequently no other satisfactory treatment option. Furthermore, a trial of vitamin K therapy, administered at a dose of 10 mg subcutaneously each day for 3 days, is also indicated if the PT is prolonged or if vitamin K deficiency is suspected.

#### *Cardiac bypass surgery*

During extracorporeal circulation, patients acquire a complex coagulopathy characterized in part by abnormal platelet function, activation of the fibrinolytic system, decreases in factor levels, and, to a lesser degree, abnormalities associated with heparin or protamine sulfate.<sup>117</sup> The acquired platelet defect involves release of alpha granule contents<sup>118</sup> and is related to platelet activation after contact with the extracorporeal apparatus. Abnormalities of platelet surface glycoproteins may also occur, possibly as a result of partial degradation by plasmin.<sup>119</sup>

Two studies<sup>120,121</sup> reported a reduction in blood loss in bypass patients treated with DDAVP. In the larger study,<sup>121</sup> Salzman and coworkers randomly administered DDAVP or placebo to patients at the completion of bypass in a double-blind study. The mean blood loss was 2210 mL per patient for 35 placebo-treated controls and 1317 mL per patient in 35 DDAVP-treated patients. The reduction in blood loss was significant and the use of DDAVP was not associated with thrombosis.

However, two randomized placebo-controlled studies<sup>122,123</sup> found that DDAVP did not decrease total

operative blood loss. In one of these reports,<sup>123</sup> there was significant shortening of the BT and a small but significant reduction in intraoperative blood loss in treated patients.

Additional support for the efficacy of DDAVP in a surgical setting is provided in a double-blind randomized trial in which DDAVP was given to patients undergoing placement of Harrington spinal fusion rods, a procedure associated with excessive blood loss.<sup>124</sup> DDAVP-treated patients had a significant decrease in bleeding compared to untreated controls.

Aprotinin, a naturally occurring inhibitor of plasmin and other serine proteases,<sup>11</sup> significantly reduces blood loss in cardiac bypass patients.<sup>125-128</sup> In one study,<sup>126</sup> 11 patients who received aprotinin during repeat open heart surgery had an average blood loss of 286 mL each, as compared with a loss of 1509 mL for each of 11 untreated controls. Only 4 of 11 treated patients required autologous transfusions, as compared with all 11 controls. Bidstrup et al.<sup>128</sup> recently summarized their studies on the use of aprotinin in cardiac bypass surgery and included a large prospective, randomized, double-blind placebo-controlled study of 77 patients having coronary artery bypass grafting. Aprotinin (administered as a 280-mg postinduction loading dose followed by a 70-mg/hr constant infusion) reduced postoperative blood loss by 50 percent in the treated group. Eight of 40 treated patients required transfusion with autologous blood, as compared with 35 of 37 controls. During bypass, there was a significant prolongation of the BT in the placebo-treated controls, but not in the aprotinin treated patients.

Aprotinin may help to preserve platelet function by preventing plasmin-induced degradation of platelet glycoprotein Ib, which is the receptor for vWf.<sup>129</sup> The use of aprotinin is not associated with clinically significant side effects.<sup>11,125-128,130</sup> Aprotinin, which is not currently licensed in the US, may play an important role in reducing the transfusion requirements associated with bypass surgery.<sup>130</sup>

#### **Conclusion**

The efficacy of hemostatic agents such as DDAVP, antifibrinolytic agents, estrogen, and vitamin K has been documented for prophylaxis and in the treatment of bleeding in patients with well-defined coagulopathies. DDAVP has almost completely eliminated the need for cryoprecipitate in patients with type I vWD. Patients with mild hemophilia A or vWD can also use intranasal or subcutaneous DDAVP in a home setting for initial treatment of a bleeding episode. The usefulness of these routes of administration would be greatly enhanced, however, if more concentrated solutions were available in the US. Pharmacologic agents can be fully utilized only if they are recommended by blood bank personnel

who understand the clinical situations in which those agents may be efficacious. With the combined efforts of blood bank personnel and physicians involved in making transfusion decisions, the use of unfractionated blood products in patients with mild bleeding disorders can be reduced or eliminated.

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# The application of restriction fragment length polymorphism mapping to parentage testing

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THE GOAL OF PATERNITY TESTING is to establish or refute the biologic responsibility of an accused man for the conception of a child. To achieve this goal, the paternity testing laboratory uses a variety of techniques to identify and evaluate objectively the distribution of selected polymorphic genetic markers in a paternity trio. From this testing, the phenotypes at a number of genetic loci of each member of a paternity trio can be ascertained and compared to determine if the alleged father could have produced the single spermatozoa that contributed to the conception of the child.

Paternity testing always results in one of two possible outcomes: either the alleged father could not have fathered the child, or he could have fathered the child. If he is excluded from consideration, the laboratory need not proceed further with the evaluation. However, if the alleged father cannot be excluded, the genetic odds ratio (or paternity index, PI) favoring the paternity of this alleged father over that of a random man of the same race is determined. The PI is calculated by accepted mathematical procedures that incorporate the respective frequencies of the paternal obligate genes in the population into a number that reflects the relative rarity or commonness of men capable of producing the desired gamete. In short, if the constellation of observed paternal genetic markers in the child is collectively rare in the population, only a limited number of men could be implicated as the father, and hence the likelihood that the accused man is the true father of the child is increased. In contrast, if the genetic constellation inherited from the biologic father is common in the population, a larger pool of men could be implicated, and the likelihood of paternity for the accused is reduced. This analytic approach constitutes the foundation upon which the field of paternity testing has been built.

Parentage testing detects and evaluates polymorphisms present in the genome that result in the production of polymorphic gene products. These products can be distinguished from one another on the basis of slight variations in biochemical properties (net charge, for example) or by serologic characteristics distinguishable by

using highly defined alloantisera. It is not the intent of this review to detail recent developments in each of these technologies considered as standard in parentage testing. Recent reviews<sup>1,2</sup> are referenced that will introduce the reader to the standard methods used to resolve paternity issues. Developments have occurred over the past several years to make the standard testing methods more specific and sensitive. Examples include the continual development of monospecific alloantisera and monoclonal antisera,<sup>3</sup> as well as the use of the fluorescence-activated cell sorters to examine zygosity questions with improved accuracy.<sup>4</sup>

Polymorphisms detected in gene products by electrophoretic or serologic techniques stem from polymorphisms in the nucleotide sequence of the respective genes encoding them. DNA probe technology enables such polymorphisms to be visualized at the DNA level. DNA probes are typically derived from the genome through molecular cloning and therefore are well-characterized molecules, available in high purity and large quantities. Their utility as molecular probes is due to the base-pairing properties of DNA which enable a single-stranded DNA probe to match, or hybridize, with a complementary, polymorphic segment of DNA residing in the genome.

One consequence of polymorphisms in the nucleotide sequence of the genome is an alteration in the spatial arrangement of restriction endonuclease recognition sites. Restriction endonucleases are enzymes normally isolated from microorganisms that will cut a length of DNA whenever a particular sequence of nucleotide bases is encountered. In general, the recognition sites for restriction enzymes consist of four to six nucleotides of specific sequence, and, to date, over 100 enzymes have been characterized, many of which are available commercially. Digestion of chromosomal DNA with a particular restriction enzyme results in the fragmentation of the chromosomes into a heterogeneous mixture of smaller fragments that can be partially fractionated by agarose gel electrophoresis.

Chromosomal DNA from two unrelated persons has different spatial arrangements of restriction enzyme sites. If these DNA samples are digested with the appropriate restriction enzyme, the different resulting patterns of restriction fragments can be visualized with a DNA probe. The technique developed to visualize differences in the

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